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Kiwifruit bacterial canker in 'Hayward' kiwifruit:

The application of observational study design and epidemiological techniques to the study of disease outbreaks affecting plant health

A thesis presented in partial fulfilment of the requirements for the degree of

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Karyn Janine Froud

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Abstract

Bacterial canker of kiwifruit, caused by Pseudomonas syringae pv. actinidiae (Psa) biovar 3, was first recorded in New Zealand in November 2010 and quickly made production of the goldfleshed kiwifruit cultivar, 'Hort16A', which is highly susceptible to Psa, no longer viable in the Bay of Plenty region. Production of the green-fleshed cultivar, 'Hayward' has remained viable but there is uncertainty around its long-term productivity. This thesis investigated aspects of Psa in commercial 'Hayward' orchards using observational studies. The aims were to: 1) quantify a change in productivity associated with disease; 2) determine the prevalence of disease in orchards; 3) identify factors that altered the initial development of disease and 4) identify factors that impact on the presence of severe disease. Severe disease was defined as 5% or more female vines in a block showing the systemic symptoms of green shoot wilt and cane dieback. To determine Psa effects on productivity historical data from 2599 'Hayward' orchards were analysed. No reduction in productivity was found until 1 year after initial detection of Psa, after controlling for other orchard inputs that affect productivity. A crosssectional survey was sent to all Psa confirmed 'Hayward' orchards and 430 growers provided information about one of their 'Hayward' orchard blocks. The survey found 84% of orchard blocks were affected by disease and 57% had green shoot-wilt and/or cane dieback reported. Blocks typically had a low within block prevalence of systemic symptoms (Median = 5% of vines). In 194 orchards that were asymptomatic at the start of the study period the probability of disease developing in a block increased in association with use of Psa protectant sprays immediately post-pruning and using artificial pollination. A lower probability of disease developing was associated with undertaking summer girdling and with the presence of older male vines. The probability of developing severe disease was investigated in 331 orchard blocks that were symptomatic. The probability increased with time after Psa was first detected in a block and was highest when frost damage occurred, when poplar, cypress or pine shelter belts were present and when artificial pollination was used. The probability of severe bacterial canker was lower when spring girdling of female vines was undertaken. The results of this study can be used to prioritise future research. The thesis has also demonstrated the utility of observational studies for plant disease research.

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Publications arising

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- Froud, K., Cogger, N., 2015b. Use of observational study designs and multivariable analysis in plant protection, In: Beresford, R., Froud, K., Worner, S.P., Kean, J. (Eds.) The plant protection data toolbox: On beyond t, F and X. Caxton, Christchurch, pp. 113-120.
- Froud, K., Cogger, N., Beresford, R., 2014. The relationship between kiwifruit bacterial canker disease (Psa-V (*Pseudomonas syringae* pv. *actinidiae*)) and kiwifruit productivity. New Zealand Plant Protection 67, 34-40.
- Froud, K., Cogger, N., Beresford, R., 2015a. Two case studies using observational study designs and multivariable analysis investigating kiwifruit bacterial blight in New Zealand, In: Beresford, R., Froud, K., Worner, S.P., Kean, J. (Eds.) The plant protection data toolbox: On beyond t, F and X. Caxton, Christchurch, pp. 121-137.
- Froud, K., Cogger, N., Beresford, R., 2016. Kiwifruit bacterial canker in 'Hayward' kiwifruit:

 Design of a quantitative questionnaire for kiwifruit growers. New Zealand Plant

 Protection 69, 30-38. (Chapter 5)
- Froud, K., Cogger, N., Beresford, R., Clark, G., 2015b. Orchardist-observed prevalence of symptoms of kiwifruit bacterial canker disease in 'Hayward' kiwifruit blocks in New Zealand. Acta Horticulturae: Proceedings of the 1st International Symposium on Bacterial Canker of Kiwifruit. 1095, 45-48. (Chapter 6)
- Froud, K., Everett, K., Tyson, J., Beresford, R., Cogger, N., 2015c. Review of the risk factors associated with kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae*. New Zealand Plant Protection 68, 313-327. (Chapter 2)

